EFFECT OF DRIED RHIZOMES OF *DORONICUM HOOKERI* ON THE COGNITIVE DEFICIT AND THE EXPRESSION OF NICOTINE WITHDRAWL SYMPTOMS IN RATS.

SYNOPSIS OF THE THESIS

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DECLARATION

I hereby declare that the dissertation synopsis entitled **"Effect of dried rhizomes of *Doronicum hookeri* on the cognitive deficit and the expression of nicotine withdrawl symptoms in rats"** to be submitted for the partial fulfillment of the Degree of Doctor of Philosophy is my original work and the dissertation has not formed the basis for the award of any degree, diploma, associate ship or fellowship of similar other titles. It has not been submitted to any other University or Institution for the award of any degree or diploma.

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List of Abbreviations and symbols

Sr. no.	Abbreviations and symbols	Full form
1.	CNS	Central Nervous System
2.	$5\text{-}HT_{2A}$	5-hydroxy-tryptamine
3.	WHO	World Health Organization
4.	VTA	Ventral tegmental area
5.	DG	Dentate gyrus
6.	mPFC	Medial prefrontal cortex
7.	NA_C	N-acetylcysteine
8.	GABA	Gamma aminobutyric acid
9.	Nic_{A2}	Nicotine degrading enzyme
10.	BNST	Bed nucleus of stria terminalis.
11.	$INAH_3$	Interstitial nucleus of anterior hypothalamus.
12.	ER	Estrogen receptors
13.	ERB	Estrogen receptors beta
14.	AR	Arteriovenous
15.	MR	Mineralocorticoid
16.	GR	Glucocorticoide
17.	IL1beta	Interleukin-1ß

18.	TNF alpha	Tumor necrosis factor alpha
19.	IFN gama	Interferon-gamma
20.	DAT	Dopamine transporters
21.	Na	Sodium
22.	Cl	Chloride
23.	SN	Substantia nigra
24.	DA	Dopamine
25.	KOP_r	Kappa opioid receptor
26.	BDNF	Brain-derived neurotrophic factor
27.	LTP	Long term potential
28.	4-FBS	4-Fluoro-N-(4-sulfamoylbenzyl) Benzene Sulfonamide
29.	TCB	Transient cortical blindness
30.	LHb	Lateral habenula
31.	TPD	Tangle-predominant dementia
32.	SCH	Selective adenosine A_{2a} subtype
33.	$PPAR_r$	Peroxisome proliferator activated receptor
34.	nAch	Nicotine acetylcholine receptor
35.	MAPK	Mitogen-activated protein kinase
36.	CRF	Corticotropin releasing factor
37.	TLC	Thin layer chromatography
38.	HPTLC	High-performance thin layer chromatography
39.	IR	Infrared
40.	NMR	Nuclear Magnetic Resonance
41.	UV	Ultraviolet
42.	CPCSEA	The Committee for the Purpose of Control and Supervision of Experiments on Animals
43.	%	Percentage
44.	^0C	Degree celsius

1. Introduction:

Nicotine is the main psychoactive component of tobacco smoke. More than one billion people smoke around the globe and it kills about eight million people every year, among these seven million death is the result of direct tobacco use.The nicotine administration is of two types:

- Acute use of nicotine administration
- Chronic use of nicotine administration

The chronic use of nicotine administration (6mg/kg/day) for 14 days or above. The use of daily nicotine administration causes chronic phase at a particular dose i.e. 6mg/kg i.p.[1] Both acute & chronic use of nicotine administration produce the nicotine effect on CNS,Generally in the 5-HT2A receptors (5-HT2ARS) and the lateral habenula (LHb) a central area in nicotine addiction that has been generally shown under a strong 5-HT2AR-modulation. Acute nicotine (2mg/kg i.p) & chronic nicotine (6mg/kg/day for 14 days) differently affected both the 5-HT2AR- immunoactive (IR) neurone number and the 5-HT2AR immunostaining area in the different brain area. These all data reveal that acute and chronic nicotine differentially affect the 5-HT2AR function in different brain area.[2][3] Generally the chronic use of nicotine results in the development of dependence and upon abstinence it produce affective and somatic withdrawl symptoms that lead to relapse,because the brain is occupied by a variety of nicotine acetylcholine receptors and range of other neurotransmitters system are affected by chronic nicotine,so it may also affect various cognitive functions i.e. sensory, motor, attention, executive function,learning and memory.[4][5]

Addiction statistics:

Tobacco addiction is the leading preventable cause of death in U.S. and is the second leading cause of death in the world. The WHO estimates that there are 1.3 billion smokers worldwide today and contributes to approximately 5 million death each year.With the present smoking trends, tobacco will kill 10 million people each year by 2020. Non smokers exposed to environmental tobacco smoke have a significantly higher risk of developing cancers and pulmonary disease and psychosis disorders. Children exposed to second, hand smoke develop a variety of respiratory disorders and morbididty.[6][7]

Table.1. Physiological changes after a person quits smoking as given below. [8][9][10]

TIME	PHYSIOLOGICAL EFFECT
20 minutes	Heart rate return to normal.
12 hrs	Carbon monoxide level in the blood returns to normal.
2 weeks to 3 months	Heart attack risks begin to drop and lung function improves.
1 year	Risk of coronary heart disease is half that of someone who smokes.
10 year	Risk of developing several types of cancer is decreased.
15 year	Risk of coronary heart disease is the same as a non smoker.

Table.2. Symptoms of nicotine addiction:

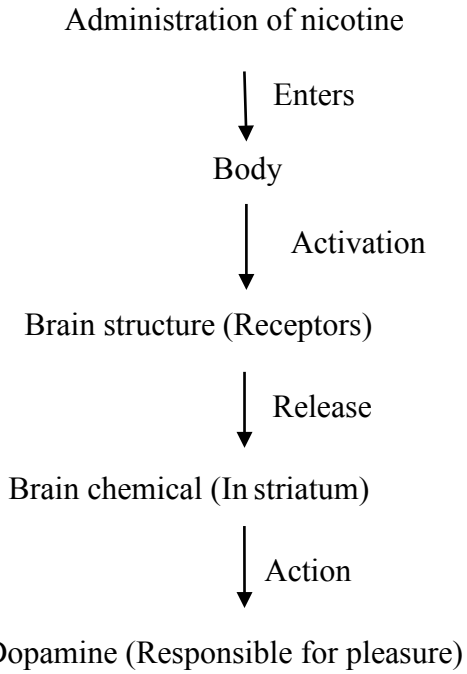
Sr.No.	SYMPTOMS
1.	Withdrawl symptoms after cessation.
2.	Smoking more than usual.
3.	Persistent desire to smoke despite efforts to decrease intake.
4.	Extensive time spent smoking or puchanging tobacco.
5.	Postponing work, social or recreational events in order to smoke.
6.	Continuing to smoke despite health hazards.

Table.3. Physical and mental status examination:

Sr.No.	Physical status	Mental status examination
1.	Physical effects of nicotine use include increased heart rate, accelerated blood pressure and weight loss.	Nicotine enhances individual's level of alertness.
2.	Physical effects of nicotine withdrawl and smoking Cessation includes weight gain due to increase in appetite, descreased heart rate.	Tobacco abuse and dependence may Stimulate afrantic, almost manic picture.
3.	Patient commonly disclose the odour of smoke , tearstained teeth and premature skin aging.	The speech may also be accelerated in line with the behaviour.
4.	Symptoms of nicotine toxicity, otherwise known as acute nicotine poisoning include nausea, vomiting.	Tobacco contribute to irritability often smoothened by a dose of nicotine.

Neuronal mechanism of action of Nicotine Addiction:

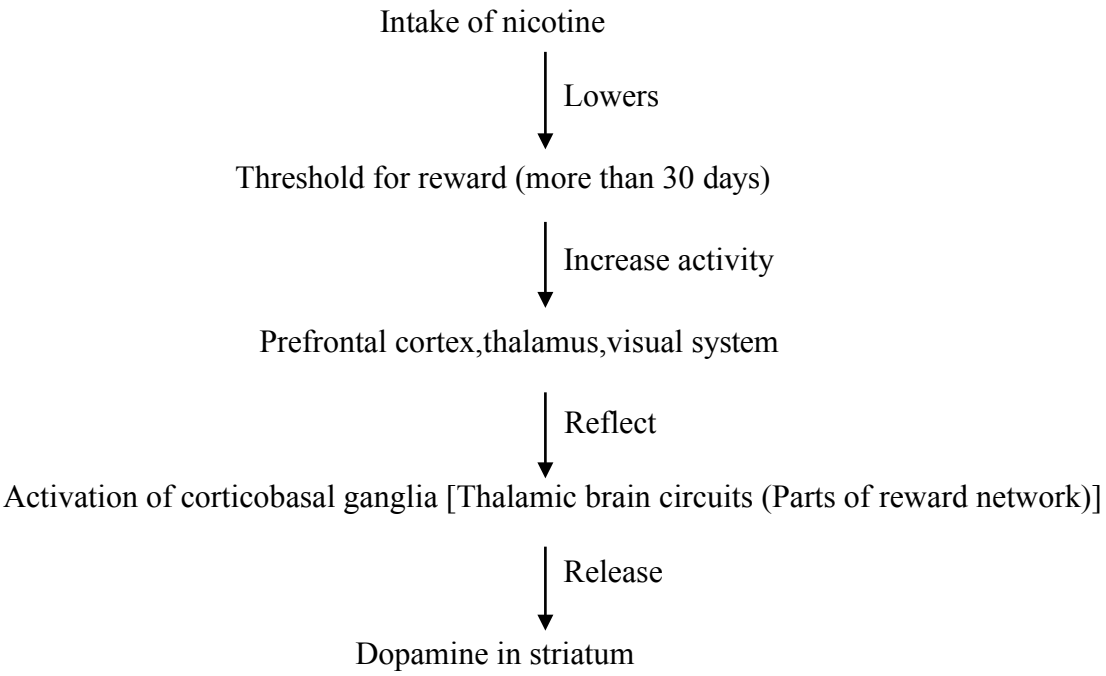
Generally nicotine is addictive because it triggers a reaction in the brain rewards system,the structures responsible for giving us pleasurable sensation,the drug intensifies the activity of the neurotransmitter dopamine in a part of brain called nucleus accumbens.[11]



Nicotine that gets into your body through cigarettes or any other routes of administration activates structures normally present in the brain called receptors.When these receptors are activated, they release a brain chemical called dopamine which makes you feel good.This pleasure response to dopamine is a big parts of the nicotine addiction process.[12] Generally nicotine addiction affect the different brain areas,brain receptors ,neurotransmitter and other enzymatic reactions which has been briefly deacribed as below:

1. **Nicotine addiction affect the brain:**

The intake of nicotine lowers the threshold for the reward systems more than 30 days and further it increase the activity in prefrontal cortex,thalamus and visual system which reflect the activation of corticobasal ganglia which is the part of the reward network which lead to the release of dopamine in striatum area of the brain.[13][14][15]



2. Effect of nicotine addiction in brain area:

Nicotine addiction mostly affect the brain area such as: Nucleus acumbens,prefrontal cortes,ventral tegumented area (VTA) ,amygdala and hippocampus respectively. Along with this it also affect the others functions such as:

- Increase 5-HT2AR-IR neurons occurs only in the hippocampal DG in nicotine acute use of administration.
- Most common nicotine affect on the change of the neuropil (5-HT2AR) expression, either a decrease in mPFC (acute nicotine) and ST(chronic nicotine) or as increase in ventral tegumented area(VTA) (acute nicotine),NAc(chronic nicotine)
- Also affect the 5-HTR subtype,the HT2A gene and 5-HTR protein, both neurons and glial cells.[16][17][18][19]

3. Effect of nicotine addiction in neurotransmitters:

Generally nicotine addiction affects the mainly following neurotransmitters such as:

- Dopamine
- GABA & Glutamate
- **Effect of nicotine addiction on Dopamine:**

When the nicotine has been administred in the body it get release a chemical named as dopamine which gives a pleasure feeling and make the cause of addiction.[20]

- **Effect of nicotine addiction on GABA & Glutamate:**

Both GABA & Glutamate play an important roles in the reinforcing effects of nicotine and development of nicotine dependence. Chronic nicotine exposure facilitates excitatory glutamate neurotransmission and repeated nicotine exposure attenuates inhibitory GABA neurotransmission.[21]

4.Effect of nicotine addiction on brain receptors:

Nicotine imitates the action of a natural neurotransmitter called acetylcholine and bind to a particular type of acetylcholine receptor,known as nicotinic receptor.Generally nicotine like Ach, is a nicotinic receptor agonist. The binding of nicotine and Ach to nicotinic receptors cause a conformational change that either opens or close the receptors ion channels,thereby it changing the receptors functional state.[22][23]

Nicotine addiction withdrawl symptoms:

The chronic use of nicotine administration causes the various affect in the brain areas.The most studies said that the commonly occur nicotine withdrawl symptoms are as follows:

- Anxiety
- Deperession
- Stress
- Dysphoria

These are the common nicotine addiction withdrawl symptoms & these are also related to the psychosis symptoms.[24]

- **Anxiety and nicotine withdrawl:**

Anxiety mainly occurs due to the disturbance in the neuronal circuitry area of brain e.g. Amygdala and limbic system structure,medial prefrontal cortex and parietal cortices. This disorder generally trigger flight or fight stress response, release flood of chemicals and hormones like adrenaline in brain. The various neurotransmitters that involves in the anxiety such as: Serotonin,GABA,Dopamine etc. play an important role in the upregulation and downregulation of anxiety.This disorder also disturb the various receptors activity such as muscarinic M1 receptors induce anxiety through nor-adrenergic pathway, nicotine facilitates GABAergic neuron that induce anxiety.[25][26]

- **Depression and Nicotine Withdrawl:**

Generally depression found in the brain area such as: Amygdala,hippocampus and dorsomedial thalamus. The main cause of this withdrawl symptoms is the influx of cortisol triggered depression and cause amgdala enlarge.This part of brain associated with emotional

response.Due to its enlargement it causes the sleep disturbance,change in activity level and changes in others hormones and also shows the abnormalities in the various neurotransmitters such as Dopamine,Norepinephrine and serotonine and also disturb the receptors functional activity i.e. Serotonin 1 A (5- HT1A) and 1B (5-HT1B) receptors.[27][28]

- **Dysphoria and nicotine withdrawal:**

Along with anxiety and depression which lead to dissatisfaction,restlessness and unhappy. This withdrawl symptoms generally found in brain area such as: amygdala,thalamus ,hypothalamus,hippocampus and prefrontal cortex i.e.Bed nucleus of stria terminalis (BNST) and 3rd interstitial nucleus of anterior hypothalamus (INAH3).This also irregulates the function of neurotransmitter e.g. serotonin and epinephrine. It also the receptors activity e.g. Estrogen receptors and genotype intraction of alpha and beta estrogen receptors and AR get decreased. In male specific genotypes intraction between ERB and AR increase add ratio of gender dysphoria.[29][30][31][32]

- **Stress and Nicotine withdrawl :**

The stress generally found the the brain area such as: Hypothalamic-pituatory-adrenal axis, amygdala and prefrontal cortex. Nicotine addiction distrupt the synapse regulation that further reduce the sociability and the avoidance of interaction with others. Generally the stress kill the brain cell,even reduce the size of brain. Chronic stress has shrinking effects on the prefrontal cortex (Responsible for memory and learning). It generally effects the functional activity of Glutamate neurotransmitter and also reduce the function of receptors such as: Mineralocorticoid (MR) and Glucocortocoides receptors (GR).[33]

- **Cognitive response and nicotine withdrawl:**

Nicotine withdrawl is associated with cognitive deficit including attention,working memory and episodic memory impairments.These cognitive deficits are a hallmark of nicotine abstinence which could be targeted in order to prevent smoking relapse.The presence of cognictive deficit correlated with microglial activation in the hippocampus & prefrontal cortex. An increased expression of neuroinflamatory markers including IL1beta,TNFalpha & IFNgama has been found in both memory related brain regions.The enhancement of TNFalpha &IFN gama plasmatic level at the same time point during nicotine withdrawl.The non steroidal anti-inflamatory drug prevent the cognitive deficit and microglial reactivity during withdrawl.[34]

- **Purpose of study:**

- Pharmacological effects of plant extract with less toxic effect against the cognitive deficit expressions occurs due to activation of neuronal glial cells in brain.
- Pharmacological effects of plant extract against withdrawl symptoms of nicotine addictions such as Anxiety, depression, stress and dysphoria.

- **Background of topic:**

Nicotine is the main psychoactive component of tobacco smoke. Chronic use of nicotine results in the development of dependence and upon abstinence it produces affective and somatic withdrawal symptoms that lead to relapse.[35] Because the brain is occupied by a variety of nicotinic acetyl cholinic receptors and range of other neurotransmitters systems are effected by chronic nicotine, so it may also affect various cognitive functions i.e. sensory, motor, attention, executive function, learning and memory . [36] Upon quitting, intense craving for nicotine,cognitive impairment, and increased anxiety has been reported in chronic smokers.During early nicotine abstinence.[37]Cognitive impairment may promote short-term smoking relapse.Animal models of nicotine withdrawal have described impaired attention performance in a variety of learning and memory tasks and novel object recognition which is parallel to working memory in humans . During withdrawal from chronic nicotine, mice showed robust deficit in contextual base fear and spatial object learning. After spontaneous nicotine withdrawal, rats failed to attend more trials than control animals in a continuous performance task.[38][39]

Plant profile:

Vrscikakanda consists of dried rhizomes of *Doronicum hookeri* C.B.Clarke is a robust herb growing in the Sikkim and Himalaya region between 3500 to 4200.

Family –Asteraceae

Regional language name:

Daarunaj-akrabi

DESCRIPTION:

a) Macroscopic: Brown irregular pieces 3 to 5 cm long and 0.2 to 0.8 cm in width; scale leaf scars present; fracture smooth, taste starchy, astringent, odour present but not specific.

b) Microscopic: TS of rhizome shows 3 or 4 layers of cork containing thin walled cells; cortex parenchymatous; vascular bundles numerous, arranged in a ring in the outer region of the cortex, each surrounded by a bundle sheath of sclerenchymatous fibres; phloem present towards the periphery and xylem towards the pith region, almost all the cells of cortex and pith are compactly filled with simple starch grains of various size ranging from about 10 to 60 μ; some cells of the cortex are filled with yellowish brown colouring matter.

c) Powder: Light yellowish brown, shows simple and compound starch grains of various sizes, about upto 60 μ and spherical, sub-spherical to ovoid in shape with a radiate hilum and very faint striations that are visible only in large starch grains; individual or groups of parenchymatous cells filled with starch grains; fibres sclerenchymatous, nonseptate, lignified with tapering ends, broad lumened, ranging from 76 to 125 μ in length; xylem vessels with spiral and reticulate thickenings; taste slightly astringent; odour present but not specific.

Constituent: Essential oil

Therapeutic uses:

- Facial pals.
- Poisoning due to bites.
- Uterine pain.
- Heart disease.
- Paralysis.
- Hemiplegia.
- Pain in the abdomen.
- Mania.
- Psychosis.
- Bubonic plague.[40][41][42][43][44]

2. Review of literature:

1. **Casarrubea et al.2021** has explored the role of the LHb by comparing the behavioral effects of short-term nicotine withdrawal in chronically treated LHb-lesioned rats. Quantitative analysis revealed increased anxiety-like behavior in chronically treated overnight nicotine-deprived rats, as manifested in reduced general and focused exploratory behaviors, which was eased in animals that received nicotine. the LHb lesion induced a stronger anxiolytic-like response to the acute challenge of nicotine in chronically nicotine-exposed animals, implicating the LHb in the anxiogenic effect of chronic nicotine exposure.[11]
2. **Zaniewska et al. 2021** has show that lorcaserin alleviated locomotor responses and depression-like state during nicotine withdrawal and propose that the modulatory effect of lorcaserin on the 'affective' aspects of nicotine cessation may be linked to the positive changes caused by the compound in hippocampal neurogenesis during nicotine withdrawal.[12]
3. **Rehman NU et al. 2020** has investigated that Anxiety like behavior has observed during nicotine withdrawal. Plasma BDNF level has reduced during nicotine withdrawal as compared to the saline group reflecting mild cognitive impairment, stress, and depression. Withdrawal from chronic nicotine altered hippocampal plasticity, caused suppression of long-term potentiation (LTP) in the CA1 area of the hippocampus. The results had showed that 4-FBS at 40 and 60 mg/kg significantly prevented nicotine withdrawal-induced cognitive deficits in behavioral as well as electrophysiological studies. 4-FBS at 60 mg/kg upsurge nicotine withdrawal-induced decrease in plasma BDNF and had conclude that 4-FBS at 40 and 60 mg /kg effectively prevented chronic nicotine withdrawal-induced impairment in long term potentiation and cognitive performance.[44]
4. **Flores et al.2020** has shown that during withdrawal, intact females displayed greater anxiety-like behavior and increases in corticosterone levels as compared to male and OVX rats. Females tested in the estrus phase (when E2 is relatively low) displayed less anxiety-like behavior and had lower corticosterone levels versus all other phases. Anxiety-like behavior and corticosterone levels were positively correlated with E2 and negatively correlated with progesterone levels. Intact females displaying high E2/low progesterone showed greater anxiety-like behavior and corticosterone levels

as compared to females displaying low E2/high progesterone. Lastly, OVX-E2 rats displayed greater anxiety-like behavior than OVX-E2+progesterone rats. These data has suggest that E2 promotes and progesterone reduces anxiety-like behavior produced by nicotine withdrawal.[26]

5. **Aleem M et al.2020** has conclude that the contemporary pharmacological studies showed the anti-microbial, antifungal, hepatoprotective, and antioxidant activity of Darunaj-aqrabi. However, most of the aspects of this herb has still not been identified. Along with this, there are inadequate details on the phytochemistry and toxicological profile. Due to the extensive traditional uses of Darunaj-aqrabi, more research on pharmacological activities, phytochemistry, toxicity and adverse effects tend to be required to determine its medicinal values.[41]
6. **Marathe P A et al.,2020** has showed benificial effects on alcohol relapse possibly by virtue of its effect on GABA and Dopamine levels in the brain reward pathway in alcohol dependent animals.
7. **Bockman et al .2018** has shown that rearing environment regulates the sensitivity to the interoceptive effects of nicotine and α4β2*-nACh receptor expression in the mesolimbic dopamine pathway.[39]
8. **Carboni et al. 2018** has revealed that the CRF transmission, in addition to its role in increasing withdrawal-related anxiety, may be involved in the development of nicotine-habituated behaviours through reduced control of impulses and the aberrant memory plasticity characterising addiction.[25]
9. **Kotagale et al. 2018** has investigated that brain agmatine analysis carried out at 72 h time point of nicotine withdrawal showed marked decrease in basal brain agmatine content as compared to control. Overall, the data has indicate that agmatine attenuates nicotine withdrawal induced memory impairment through modulation of α2adrenergic receptors. Thus, agmatine might has therapeutic implications in the treatment of cognitive deficits following nicotine withdrawal.[34]
10. **Qi et al. 2014** has shown the overexpression of CRF did not affect baseline ICSS thresholds but diminished the elevations in ICSS thresholds associated with precipitated and spontaneous nicotine withdrawal. The real-time reverse transcriptase (RT)-PCR analysis showed that the overexpression of CRF led to a decrease in CRF1 mRNA levels and an increase in CRF2 mRNA levels in the CeA. In conclusion that overexpression of CRF in the CeA diminishes the dysphoric-like state associated with

nicotine withdrawal and this might be driven by neuroadaptive changes in CRF1 and CRF2 receptor gene expression.[27]

11. **Syed S N et al. 2014** has investigated that this study demonstrating the effectiveness of Doronicum hookeri in hepatotoxicity. The study has concluded that the ethanol extract in a dose of 500 mg/kg offered a partial hepatoprotection (36%) against toxicity induced by carbon tetrachloride in rats ($p<0.01$). The study also showed good antioxidant activity of 500mg/kg ethanol extract in in vivo (GSH $p<0.001$) and in vitro tests. While the ethanol extract of 300 mg/kg has less effective, the aqueous extracts (300and 500 mg/kg) showed statistically not significant hepatoprotective and antioxidant activity. Further studies are required for isolation and characterization of the active principle, which may serve as a lead for the development of new antioxidant hepatoprotective agent.[14]
12. **Gupta D et al.2011** has revealed that *Doronicum hookeri* Hook f.roots has extracted with two solvents of different polarity and evaluated for their in vitro antioxidane activities of the two extracts,methanolic extract possessed higher phenolis content and thus higher free radical scavenging and reduce activities.In DPPH (2,2,diphenyl-1-picryhydrazy) radical scavenging assay,methanolic extract showd scavenging similar to the standard BHT (butylated hydroxytoluene)(85%) at con. O.5mg/ml. Methanolic extract also exhibited more that 905 inhibition to ABTS radicals at conc. above 0.3 mg/ml. Reducing power activity of methonolic extract has also higher than dichloromethane extract.Dichloromethane extract has also higher than dichloromethane extract had rich in flavonoids and showed considerable metal chelating (78.684 -0.695% at 0.5 mg/ml),nitric oxide (52.232-0.934% at 0.15 mg/ml) and superoxide radical (59.882+0.772% at 0.5 mg/ml) scavenging activities.[11]
13. **Indian Ayurvedic Pharmacopoeis,1st edition.2008** has show the traditional use of *Doronicum hookeri* in psychosis symptoms.
14. **Verma K. et al., 2007** has revealed the antifungal activity of *Doronicum hookeri* Hook. f. Methanolic and Dichloromethane extract of dried root shows antifungal activity against *Saccharomyces cervisiae* and *Candida albicans* at a concentration 500 to 1000 µg/ml**(MIC).**
15. **Kumar V. P. et al., 2006** has revealed the antibacterial and antifungal activity of *Doronicum hookeri* Hook. f. The mixture of dichloromethane and methanol extract of dried roots shows broad-spectrum antibacterial.

Rational:

As per the literature review, it was found that current treatment on the expression of nicotine withdrawl symptomes are potent but had a lot of adverse and side effects. So, a new drug/formulation is required with maximum potency and a negligible side and adverse effect. As the present study comprises of natural origin drug which may have less side effect with maximum potency. From various kinds of literature, However no such experimental work has found on nicotine withdrawl symptoms of dried rhizomes of *Doronicum hookeri* and this is the noval plant So, experimental work is required for ensuring the abolishing the expression of nicotine withdrawl symptomes.

3. Aim of study:

Effect of dried rhizomes of *Doronicum hookeri* on the cognitive deficit and the expression of nicotine withdrawl symptoms in rats.

Objective of study:

1. Phytochemical screening of *Doronicum hookeri.*
2. Characterization of various extracts of *Doronicum hookeri.*
3. In-vivo screening of plant extract on the cognitive deficit.
4. In-vivo screening of plant extract on the expression of nicotine withdrawl symptomes.
5. To determine the BDNF profile.
6. To determine the conc. of dopamine level.
7. To determine the conc. of GABA level.

4. Proposed Research Methodology:

1. **Literature survey:** The literature collects from the review, papers, books, and Google Scholar, PUBMED, MEDLINE, NCBI, Science Direct.
2. **Selection of plants:** As per the literature review, it was found that current drugs for the treatment of nicotine withdrawl symptomes are potent but had a lot of adverse and side effects. So, a new drug is required with maximum potency and a negligible side and adverse effect. As my study comprises of natural origin drug which may have less side effect with maximum potency. From the latest literature, it was found that till now no experimental work had done on the dried rhizome of *Doronicum hookeri* on the cognitive deficit and the expression of nicotine withdrawl symptoms. Hence this is a noval plant for this study so I have selected this plant for this study respectively.
3. **Collection of plant material:**

The plant will be collected from the region of himalyas.

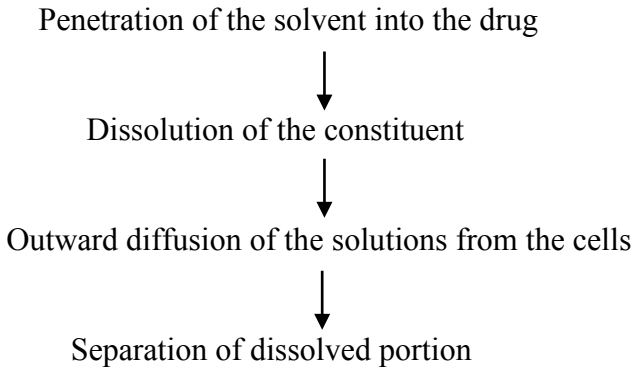
4. **Extraction of dried rhizome of *Doronicum hookeri*:**

The extraction will be done by multiple extraction methods and these methods has been listed below:

1. **Soxhlet-Extraction**
2. **Maceration at laboratory tempt.**
3. **Higher tempt. extraction**
4. **Fluidized-bed extraction**
5. **Micowave-assisted extraction**
6. **Accelerated solvent extraction**

Extraction in chemistry is a separation process used to separate a substance from a matrix**.** In recent years active principles from plants have been isolated or obtained as purified products of precisely known potency and stability.The plant contains chemical substances some of which provide relief from a variety of diseased conditions. The solvent used for extraction is known as “Menstruum” and the undissolved residue left behind after the process is called “Marc”.

The process of drug extraction is given below:


Penetration of the solvent into the drug
Dissolution of the constituent
Outward diffusion of the solutions from the cells
Separation of dissolved portion

Process of extraction.

In the present study, the extraction will be done with the help of multiple extraction for better results.[43]

1. **Preliminary phytochemical screening of different extracts:** This plant extracts used as a test solution . [44][45]
2. **Characterisation of various extract of *Doronicum hookeri:***

To find the characterization of various active components from this plant will be done with the help of following separating techniques which has been listed below:

- **TLC**
- **HPTLC**
- **IR**
- **NMR.** [46]

3. **In-vivo screening of plant extract:**

Animals: Experimental protocols and procedures will be approved by the Institutional Animals Ethics. The care and handling of the animals are in accordance to the CPCSEA guidelines for laboratory animal use and care, Government of India.

Animal Husbandry Conditions:

The animals will be housed under controlled temperature (24 ± 2 ^{0}C) and relative humidity (45% ± 10%).

Table 4: Animal Husbandry Conditions

Temperature	24 ± 2 ^{0}C.
Relative humidity	45% ± 10%.
Air changes	10 to 15 air changes per hour.
Housing	Standard polypropylene rat cages with stainless steel top grill were used to house the animals. The cages were washed, clean paddy husk was used as the bedding material.
Sanitation	Bedding material will be changed on alternative days.
Light/dark cycle	12-hourly.
Feed & water	Standard pelleted feed and Filtered water was provided ad libitum. The diet and water were routinely analyzed for any contaminants that could reasonably by expected to affect the purpose or integrity of the study.

Preparation of the test substance : The test substance will be calculated on the basis of mg/kg .

Pharmacological evaluation of extracted compound of dried rhizome of *Doronicum hookeri*:

Animals:

Male Rats (n = 6 per group) in the weight range of 200—220 g will be used and the Animals will housed in standard plastic cages in a room maintained at temperature (24 ± 2 ^{0}C) under 12/12-hr light-dark cycle. Food and water will provided ad libitum.

Drugs:

Nicotine will purchased from the market and dissolved in 0.9 % normal saline, whereas the test drugs will prepared according to mg/kg. The solutions and test drug suspansion will be freshly prepared daily. Nicotine will be administered as a free base at a dose of 3.8 mg/kg/day in 04 divided doses through subcutaneous (sc) injection and the test drugs suspensions will be prepared in different doses (mg/kg for oral administration). All the doses will administered acutely in a volume of 5 ml/kg body weight of rats.

Induction of nicotine dependence and withdrawal:

Nicotine at the dose of 3.8 mg/kg will be administered subcutaneously in four divided doses regularly for 14 days for the induction of nicotine dependence (n= 6).After the induction of nicotine dependence, spontaneous nicotine withdrawal will be induced by quitting nicotine administration and after 72 h various behavioral, biochemical, and the level of dopamine and GABA in midbrain, striatum and cortex regions of rats brain will be performed.

Experimental Protocol: To evaluate the effect of this test substance on nicotine addiction withdrawal symptoms animals will be divided into 6 groups (n = 6 in each group) including Normal group, disease group, Standard drug group, test substance-1 and test substance-2 and test substance-3(mg/kg).

Table.5.Treatment groups for nicotine withdrawl symptomes

Groups	Drugs	Dose	Rats/group n=6/group/assay
G1	Normal distilled water	2ml	6
G2	Nicotine	3.8mg/kg	6
G3	Standard drug	mg/kg	6
G4	Test-1	mg/kg	6
G5	Test-2	mg/kg	6
G6	Test-3	mg/kg	6

After 72 h of nicotine withdrawal, various Physical parameters ,behavioral tests will be performed to evaluate the effect of this test substance. The physical parameters including water intake and food intake will be performed daily after the spontaneous nicotine withdrawl. One hour before each behavioral experiment these test substances will be administered and behavioral parameters will be recorded by using the Various animal models including:

- Morris water maize (Spatial learning and memory)
- Novel object recognition test(Memory retension)

- Inhibitory passive avoidance test(Learning & memory)
- Open field test(Anxiety related behavior)

and then will evaluated. Immediately after behavioral experiments animals will be sacrificed and blood samples will be collected for BDNF profiling after this procedure whole brain will be dissected out quickly. The whole brain will be weighed and transferred to ice-chilled perchloric acid (0.1 M) to collect samples for dopamine assay and 0.1 M phosphate buffer (pH 7.4) for GABA assay. The brain parts such as midbrain, cortex and striatum will be separated and weighed and after that dopamine assay using LC-MS-MS & GABA assay using ELISA will be performed accordingly for the final evaluation of of the concentration of dopamine and GABA in mid brain, striatum and cortex regions.These evaluation will be expressed in ng/gm respectively.[47][48][49]

Parameter assessments: During study protocol different parameters will be assessed as Physical assessments, behavioural assessments, biochemical assessments and dopamine assay and GABA assay .

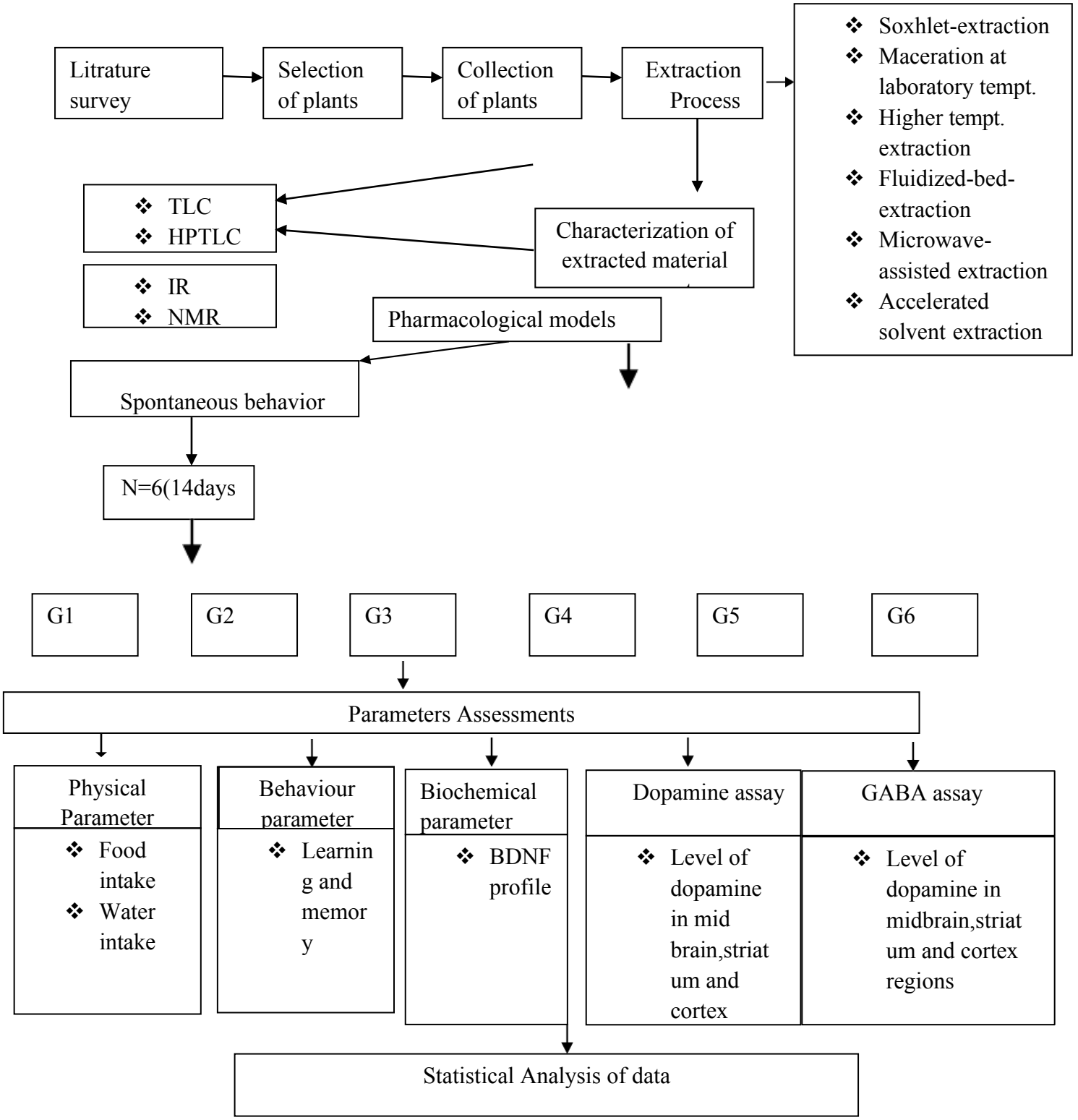
Table.7. Parameters to assessed

S.No.	Parameters
1	**Physical assessments:** ➢ Food intake ➢ Water intake
2	**Behavioral assessments:** ➢ Learning and memory assessment ➢ Locomotor activity
3	**Biochemical analysis:** ➢ BDNF profile
4	**Neurotransmitters conc. in brain regions** ➢ Dopamine assay and ➢ GABA assay

1. **Statistical Analysis of data.**

All data will be expressed with the help of Statcistical Software Graph Pad Prism-8.

5. Tentative chapter plan:



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